Treatment of Haemophilia B with purified factor IX (PPSB)

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In 1947, PAVLOWSKY was the first to differentiate between haemophilia A and haemophilia B[13]; type B is found in nearly 20 per cent of all cases[4] and is characterized by absence of a clotting factor indicated as Factor IX upon the advice of the International Committee on Haemostasis and Thrombosis.

The treatment and prevention of haemorrhages in haemophilia B is in principle the same as that in haemophilia A. Substitution of the lacking clotting factor can be, however, effected with old as well as with fresh plasma, and even with serum, because Factor IX differs from Factor VIII in that it is hardly affected by ageing or clotting of plasma.

As a result of a controlled substitution treatment in three haemophilia B patients, we reported in 1961 that adults suffering from severe haemophilia B should receive, after an initial loading dose of 2-3 l, daily transfusions of 1-2 l ACD plasma in order to attain and maintain a Factor IX concentration of 25 per cent, which is the minimum required for normal haemostasis in the event of major surgical interventions or after severe injury[8]. At the time we stated that this can be achieved only with intensive exchange transfusions unless plasma fractions are available with a considerably higher Factor IX concentration than plasma.

In the past five years we have had occasion to gain experience in five other haemophilia B patients with a preparation obtained from human plasma which contains a Factor IX concentration 50-150 times the normal, as calculated on a protein basis. This is the so-called PPSB[2,15,16]*.

General rules governing substitution of haemophilia B have accrued from this experience.

METHODS

Factor IX assay. The Factor IX assay was carried out by the same one-stage method as that described in our report on the treatment of haemophilia A with cryoprecipitate[12]. Only two modifications were required. Pooled normal plasma: from each of 30 individual subjects in good health (sex ratio 1, mean age about 30), 40 ml blood was collected in a 50-ml plastic tube containing 0.8 ml 0.55 M sodium citrate, thoroughly mixed before being centrifuged at 20,000 x *g* for 30 minutes at 4°C, and stored in 1-ml portions at −25°C. This procedure takes 4-5 hours. Factor IX-free plasma: from a patient with severe haemophilia B (<1 per cent Factor IX), known to possess no circulating anticoagulant.

The experimental error, expressed as coefficient of variation of the Factor IX activity, amounted to 6-9 per cent of the result obtained. For the special measures taken in assessing the Factor IX of PPSB, see below.

Assay of Factors II, VII and X (according to Loeliger and Koller[7]) As in assessing Factor IX, the concentration of the clotting factor in question was related to its concentration in pooled normal plasma, in dilutions chosen so that extrapolation in the calculation of concentrations is avoided.

Acquisition of PPSB. For the patients with mild haemophilia B we acquired an amount of PPSB corresponding to 10 l fresh pure plasma in terms of Factor IX activity; for the patient with severe haemophilia B the amount corresponded to 20 l fresh pure plasma. Expressed in number of flasks, these amounts were 50 and 100 flasks, respectively (the contents of one flask

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*PPSB is the abbreviation of *P*rothrombin (Factor II), *P*roconvertin (Factor VII), *S*tuart factor (Factor X) and antihaemophilic factor *B* (Factor IX). It was kindly placed at our disposal by Dr. Soulier and his co-workers of the "Centre National de Transfusion Sanguine", Paris. The generous cooperation of the Paris group is gratefully acknowledged.

average the equivalent of about 200 ml fresh pure plasma). The stock was stored at –25 °C until used. In the fifth patient, treatment was started with the emergency stock (10 flasks) and continued with a batch ordered by telephone, which arrived within 24 hours. Per patient we used only one batch, if possible, because the contents of flasks of the same batch of PPSB have the same activity.

PPSB assay. For the *in-vitro* assay we started with 0.3 ml of the contents of a flask of PPSB dissolved in 10 ml sterile distilled water. To this we added 5.65 ml citrated buffer (1 part 0.11 M sodium citrate and 4 parts 0.05 M veronal buffer prepared according to MICHAELIS, pH 7.4) and, to neutralize the heparin contained in PPSB (1 mg per flask), 0.05 ml protamine sulphate (diluted with buffer: 1 part protamine sulphate at 10 mg/ml and 14 parts buffer). Of this 1/20 diluted stock solution of PPSB (10 ml per flask) we made dilutions of 1/20, 1/40 and 1/80 in buffer, and assessed against normal plasma in dilutions of 1/10, 1/20, 1/40 and 1/80.

The Factor IX activity thus assessed *in vitro* corresponded well for all batches with the data supplied by the "Centre National de Transfusion Sanguine" (Table II).

In order to prevent unnecessary complications as a result of PPSB toxicity (e.g. pyrogenic effects), we tested *in vivo* as well. This was done in one of our adults with severe haemophilia B, patient I 753/60, whom we described in a previous publication[8] and who volunteered for this test.

To avoid unnecessary risks, the contents of one flask were dissolved not in 10 ml distilled water as prescribed, but in 90 ml physiological saline, and administered by a 30-minute intravenous drip. Immediately after this, and again 2 hours later, the Factor IX activity in the test patient was assessed, and a thrombelastogram made. The patient's body temperature was recorded continuously for 3 hours after the infusion.

The Factor IX activity was found to have increased by 2-5 per cent and the thrombelastographically measured clotting time (r-time) was 20-30 minutes (it had exceeded 60 minutes before the infusion). In the three batches thus assessed (nos. 222, 226 and 254) no side effects were observed.

In patients I 859/65 and I 125/66, who had both received many transfusions, the activity of PPSB was likewise assessed *in vivo* in order to rule out a circulating anticoagulant; temperature and condition were of course kept under observation.

Mode of administration of PPSB. The administration of PPSB after the loading dose was kept as constant as possible. The 4 adults received PPSB by continuous drip, and the 4-year-old boy received one daily infusion of PPSB in 80 ml physiological saline for about 30 minutes.

In patient I 276/64 a Braun pump was used to ensure optimally constant drip; in patient VK 420/56 we exclusively used a mechanical regulator; a Marlow infusion pump (model MHRE) was used in patients I 859/65 and 125/66. Owing to the small capacity of the Braun pump patient I 276/64 received infusions of undiluted PPSB; patient VK 420/65 was given highly diluted PPSB (contents of one flask in 500 ml glucose/NaCl 0.45 per cent/0.1 per cent). Patients I 859/65 and 125/66 received their daily dose in 300-500 ml physiological saline.

PATIENTS

Patient I 276/64 was a 25-year-old labourer, cousin of patient HM 75/61 described by us in 1961[8]. On the basis of the body weight (70 kg for a height of 170 cm) the *plasma volume* was calculated as 2.8 l.

This patient is a member of a very unusual haemophilia B family in which the severity of the clotting disorder considerably diminishes with increasing age[6]. Haemorrhages at the joints occur only during the first few years of life. The Factor IX concentration is about 1 per cent in infants, 10-20 per cent in young males and in an older patient about 40 per cent.

Preoperative values between 13 per cent and 20 per cent were found in our patient. At age 2, he had a muscular haemorrhage in the left calf, whereupon he developed *talipes equinus* which gave him great physical and mental discomfort. At the patient's urging, tenotomy of the Achilles tendon was performed on 5th March 1964. For us this was the first operation of some importance under PPSB protection. The operation was performed under general anaesthesia, with surgical haemostasis without electrocoagulation. The postoperative course was completely uneventful. The results of the substitution therapy are presented in fig. 1 and Tables I, II and III.

Patient VK 420/65 was a 25-year-old woman in good health, of normal constitution and with a body weight of 65 kg for a height of 172 cm. Although personal history and family history yielded no clue suggestive of familial haemophilia B, the patient was probably a carrier of haemophilia B with a relatively low Factor IX concentration (15-25 per cent). Since her childhood this patient had shown the typical characteristics of the mild type of haemophilia, which most physicians treating her had belittled.

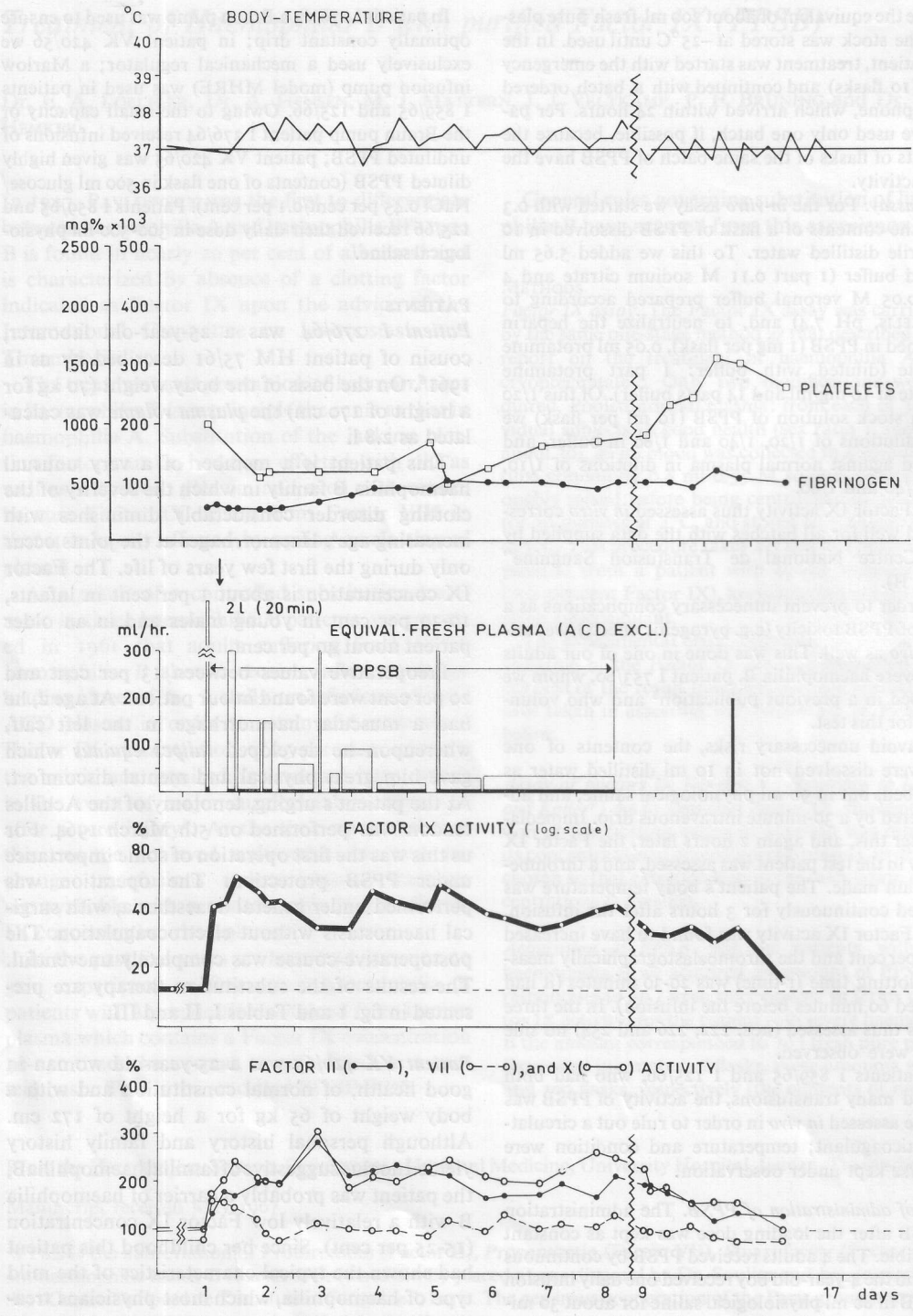

Fig. 1. Clinical data obtained during substitution therapy of patient I 276/64: operation for *talipes equinus*.

TABLE I: FLOW-SHEET OF DAILY AMOUNTS OF FACTOR IX TRANSFUSED[1].

Time after operation (days)	0[2]	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th
Patient I 276/64	2700	1800	1170	1350	630	450	720	720				400		
	PPSB batch 160										*plasma*			
Patient VK 420/65	1200	800	800	800	600	600	300	600	600	600	400	400	400	400
	PPSB batch 222				*PPSB batch 226*						*plasma*			
Patient I 859/65	2700	1800	1800	1800	1200	1200	1200	520	760	720	700	550		
	PPSB batch 226									*plasma*				
Patient I 125/66	3240	1800	1260	1260	1080	1080	720	720	720	720	720	800	800	800
	PPSB batch 254											*plasma*		
Patient[3] HM 1795/66	1200	510	420	420	420	420	400[4]							
	226			*PPSB batch 260*										

[1] Expressed as equivalents of ml fresh normal plasma (ACD excluded).
[2] 0 is the day of operation.
[3] Patient HM 1795/66 received transfusions intermittently i.e. once daily at a 24-hour interval.
[4] Half of this portion belonged to batch 254.

TABLE II: COMPARISON OF FACTOR IX ACTIVITY[1] IN PPSB FLASKS

Batch no.	*Paris*[2]	*Leiden*[3]
107	160	220
160	200	180
222	180	200
226	300	300
254	220	180
260	120	210
mean of all batches	200	215

[1] Factor IX activity expressed in equivalent amount of pure normal pasma.
[2] Assessed *in vitro* using a two-stage method. The values are 0.8 the amount indicated on the fflasks, due to the content of ACD.
[3] One-stage method.

The patient had almost fatal haemorrhages both after a tonsillectomy and later after a vaginal lesion of little significance*.

The patient's first pregnancy occurred in 1964. On 16th April 1965 she was admitted to the Obstetrical Department of the Leiden University Hospital, where a healthy daughter was born (physician in charge: Dr. J. Bennebroek Gravenhorst; then head of the department: Prof. Dr. A. J. M. Holmer). Although the perivaginal connective tissue was found thickened as a result of the gynaecological haemorrhage in 1963, parturition was free from complications and there were only some superficial ruptures of vaginal epithelium. A fact contributing to the favourable course of the parturition was the relative smallness of the child's head (the neonate weighed 2970 g). The umbilical cord blood had a Factor IX activity of 110 per cent. The maternal Factor IX activity immediately *post partum* was 35 per cent. In spite of this relatively high Factor IX concentration (with a haemostasis pattern otherwise normal for the end of pregnancy), this woman developed a moderate atonic afterhaemorrhage and a rapidly growing vulvar haematoma a few hours after parturition. Moreover, the superficial vaginal ruptures bled so copiously that the patient required blood transfusions. Since the bleeding did not stop, PPSB treatment was started about 10 hours *post partum* (fig. 2 and Table I). The haemorrhage was arrested almost immediately. The puerperal period ran a normal

*Dr. C. Merskey, Albert Einstein College of Medicine, Yeshiva University, New York U.S.A., diagnosed Factor IX deficiency on the basis of the latter haemorrhagic complication.

TABLE III: FACTOR IX ACTIVITY FOUND AFTER TRANSFUSION

time after transfusion (days)	*Patient I 276/64*		*Patient I 859/65*		*Patient I 125/66*	
	f[1]	*c*	*f*	*c*	*f*	*c*
after loading dose			34	27		
day 0	26	36	13	11	65	74
1	33	37	29	35	47	48
2	22	24	29	35	42	34
3	24	27	27	35	35	34
4	24	13	27	24	35	29
5	23	10	22	24	30	29
6	17	15	23	24	26	19
7	20	15			23	19
8					22	19
9					20	19
10					16	19
Mean	24	22	25	27	45	43

[1] f: Factor IX activity as found *in vivo*.
c: Factor IX activity as calculated from theoretical considerations. For the two patients suffering from the mild type of haemophilia B, under f the pretransfusion value is subtracted from the value found, and under c the increase of Factor IX activity as expected from the PPSB infusion is given. In calculating the expected values (c) the amount transfused during the day preceding the day under consideration is not taken into account.

course and the patient was discharged in good condition 14 days *post partum*.

Patient I 859/65 was a 28-year-old technician suffering from mild haemophilia B (Factor IX concentration 10-15 per cent), of otherwise normal constitution and in good health. On the basis of the body weight (75 kg for a height of 177 cm), the *plasma volume* was estimated as 3 l.

In this patient the haemophilia first manifested itself on the occasion of a tonsillectomy at age 2 (the diagnosis was made by Dr. Ch. H. W. LEEKSMA, Haematologist, Municipal Hospital Zuidwal, The Hague).

On several occasions since, the patient has had severe haemorrhages following accidental injuries and tooth extractions. In 1958 he underwent an operation of the patella, and the subsequent period of mobilization was complicated by severe haemorrhage from the surgical wound, and infection. The knee-joint was consequently completely ankylosed in extension. At the same time a very troublesome right-sided *talipes equinus* developed, which at the patient's request was corrected by tenotomy of the Achilles tendon. This was done under local anaesthesia on 29th June 1965. PPSB protection at this operation ensured normal haemostasis and entirely normal wound healing despite the use of a thermocauter and adrenaline administration. The patient was discharged 14 days after the operation. For data obtained in this case during PPSB therapy we refer to fig. 3 and Tables I, II and III. Two months after the substitution therapy the patient developed typical serum hepatitis, from which he recovered completely.

Patient I 125/66 was a 19-year-old student suffering from severe haemophilia B (blood Factor IX concentration <1 per cent), but otherwise in good health. On the basis of determination of the blood volume with Cr^{51} and of the haematocrit value, the *plasma volume* was calculated to be 2.2 l.

This patient had a typical history with recurrent haemorrhages at the joints and unmistakable if mild arthritis of various large joints. A muscular haemorrhage in the left forearm was followed by Volkmann's atrophy and after a haemorrhage in a calf (1965) he developed *talipes equinus* of the left foot which handicapped him severely and for which he underwent an operation under PPSB protection on 6th January 1966. The postopera-

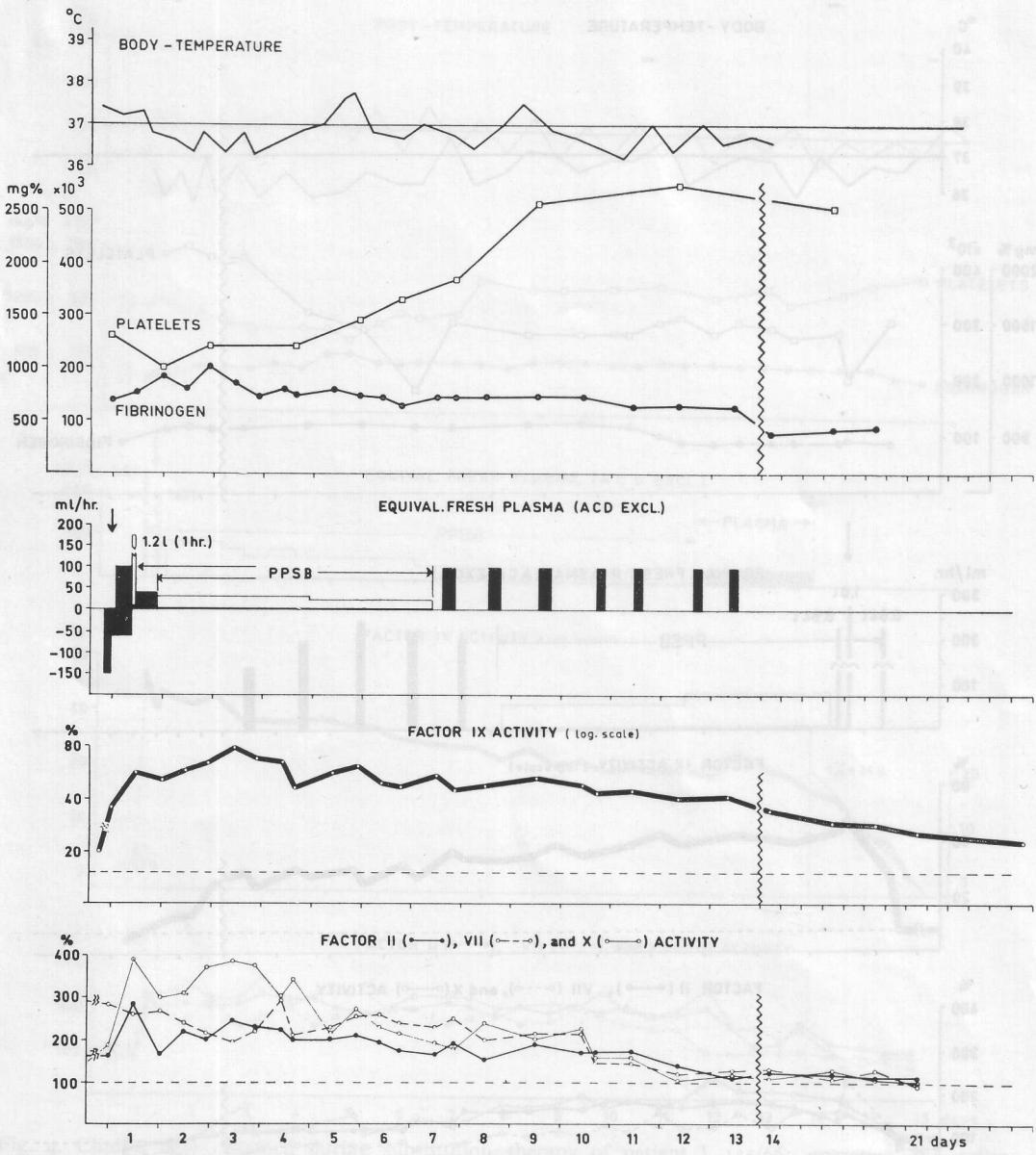

Fig. 2. Clinical data obtained during substitution therapy of patient VK 420/65 during the first 14 days *post partum*.

tive course was uneventful. Coagulation data are presented in fig. 4 and Tables I, II and III. This patient, too, developed hepatitis from which he recovered completely. Since this hepatitis occurred more than 7 months after the operation and since several blood transfusions were given during this period, a causal relation to substitution therapy is doubtful.

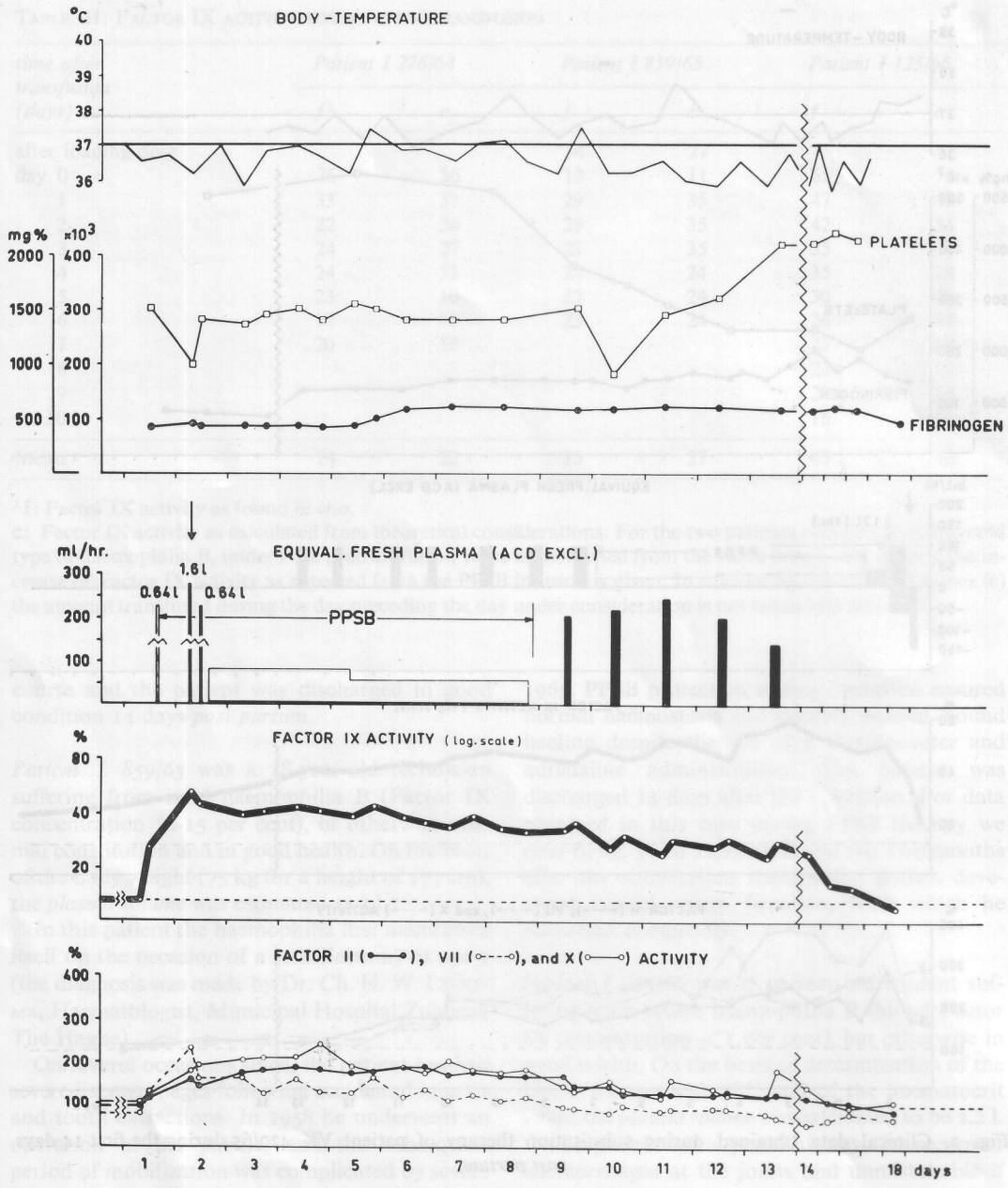

Fig. 3. Clinical data obtained during substitution therapy of patient I 859/65: operation for *talipes equinus*.

Patient HM 1795/66 was a 4-year-old boy suffering from a moderate type of haemophilia B (Factor IX concentration 1-3 per cent of normal). He weighed 16 kg and the *plasma volume* was estimated as 0.64 l. This patient is a nephew of the previously described patient I 740/60[8].

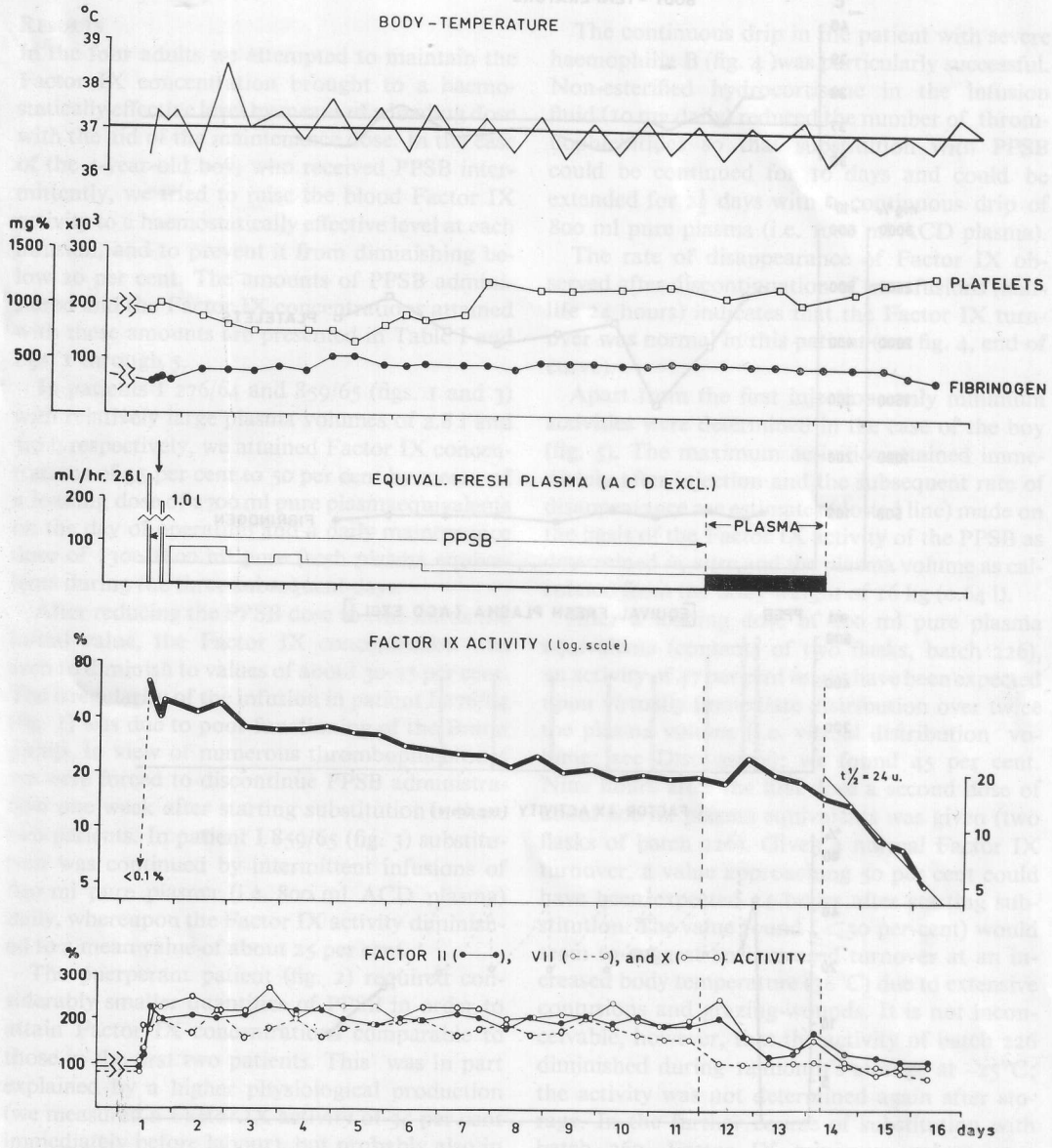

Fig. 4. Clinical data obtained during substitution therapy of patient I 125/66: operation for *talipes equinus*.

He was admitted to the surgical Department of the University Hospital $2\frac{1}{2}$ hours after a traffic accident in which he sustained a lacerated wound of the head and perhaps a cranial base fracture (periorbital haematoma, suspicious X-rays).

Partly in view of contusions of internal organs (violent abdominal pain), intensive substitution therapy with PPSB was instituted. The patient was discharged in good condition 10 days after admission, and the further course was uneventful also. The results are shown in fig. 5 and Table I.

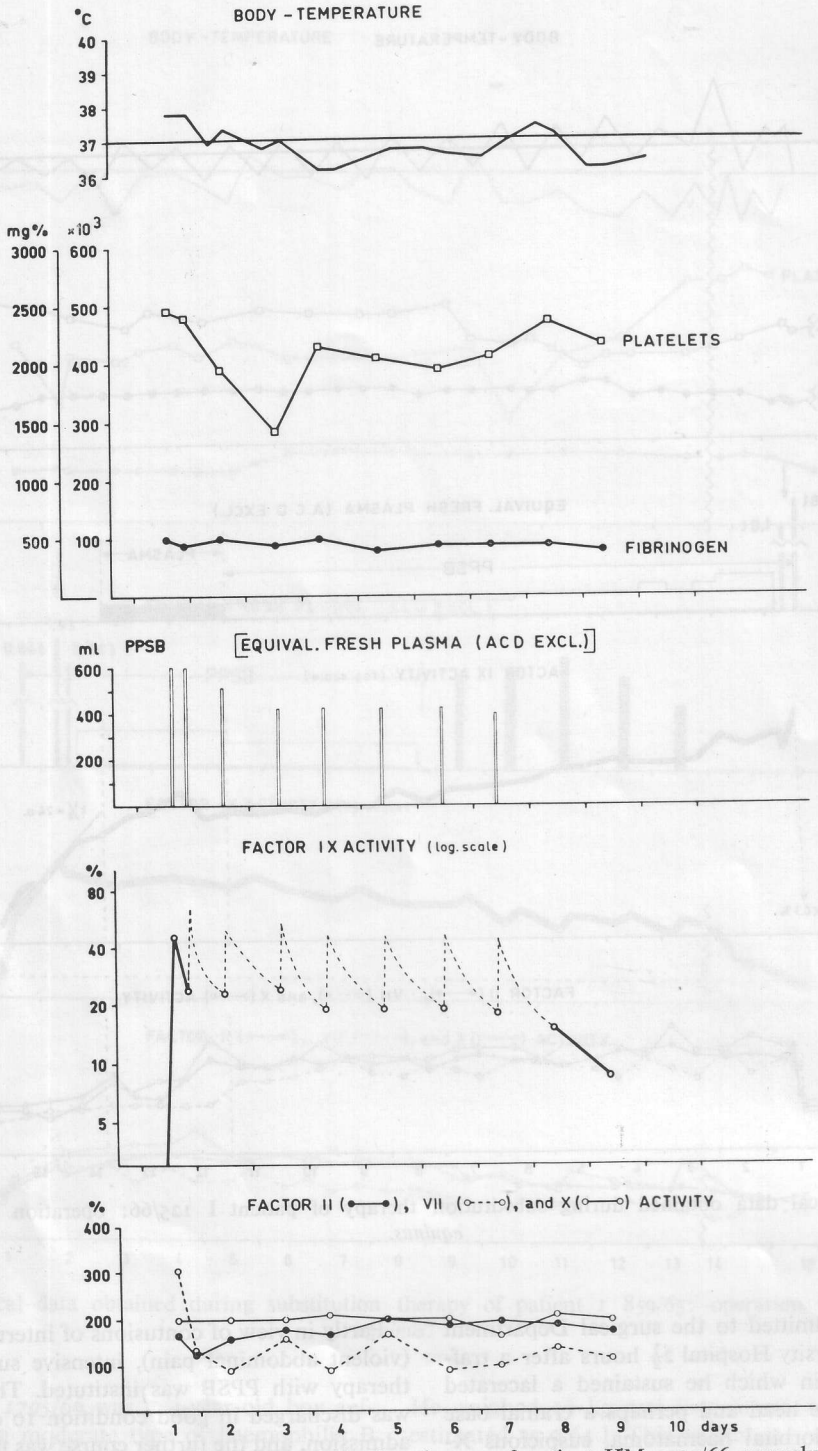

Fig. 5. Clinical data obtained during substitution therapy of patient HM 1795/66, severely injured in a motoring accident.

Results

In the four adults we attempted to maintain the Factor IX concentration brought to a haemostatically effective level by means of a loading dose with the aid of the maintenance dose. In the case of the 4-year-old boy, who received PPSB intermittently, we tried to raise the blood Factor IX activity to a haemostatically effective level at each infusion, and to prevent it from diminishing below 20 per cent. The amounts of PPSB administered and the Factor IX concentrations attained with these amounts are presented in Table I and figs. 1 through 5.

In patients I 276/64 and 859/65 (figs. 1 and 3) with relatively large plasma volumes of 2.8 l and 3.0 l, respectively, we attained Factor IX concentrations of 35 per cent to 50 per cent by means of a loading dose of 2700 ml pure plasmaequivalents on the day of operation and a daily maintenance dose of 1300-1800 ml pure fresh plasma equivalents during the three subsequent days.

After reducing the PPSB dose to two-thirds the initial value, the Factor IX concentration was seen to diminish to values of about 30-35 per cent. The irregularity of the infusion in patient I 276/64 (fig. 1) was due to poor functioning of the Braun pump. In view of numerous thrombophlebitides we were forced to discontinue PPSB administration one week after starting substitution in these two patients. In patient I 859/65 (fig. 3) substitution was continued by intermittent infusions of 640 ml pure plasma (i.e. 800 ml ACD plasma) daily, whereupon the Factor IX activity diminished to a mean value of about 25 per cent.

The puerperant patient (fig. 2) required considerably smaller quantities of PPSB in order to attain Factor IX concentrations comparable to those in the first two patients. This was in part explained by a higher physiological production (we measured a Factor IX activity of 35 per cent immediately before labour), but probably also in part by retarded degradation of the transfused Factor IX. The slow disappearance of Factor IX activity after discontinuation of substitution points in the same direction. In view of the pregnancy, the plasma volume cannot have been significantly smaller than that in the other patients; the increase in Factor IX activity from 35 per cent to 55 per cent after a booster dose of 1200 ml pure fresh plasma equivalents, indicates a plasma volume of about 3 l (see Discussion).

The continuous drip in the patient with severe haemophilia B (fig. 4)was particularly successful. Non-esterified hydrocortisone in the infusion fluid (10 mg daily) reduced the number of thrombophlebitides so that substitution with PPSB could be continued for 10 days and could be extended for $2\frac{1}{2}$ days with a continuous drip of 800 ml pure plasma (i.e. 1000 ml ACD plasma).

The rate of disappearance of Factor IX observed after discontinuation of transfusions (half-life 24 hours) indicates that the Factor IX turnover was normal in this patient (see fig. 4, end of curve).

Apart from the first injection, only minimum activities were determined in the case of the boy (fig. 5). The maximum activities attained immediately after injection and the subsequent rate of disappearance are estimates (dotted line) made on the basis of the Factor IX activity of the PPSB as determined *in vitro* and the plasma volume as calculated from the body weight of 16 kg (0.64 l).

After a loading dose of 600 ml pure plasma equivalents (contents of two flasks, batch 226), an activity of 47 per cent might have been expected upon virtually immediate distribution over twice the plasma volume (i.e. virtual distribution volume; see Discussion); we found 45 per cent. Nine hours after the first dose a second dose of about 600 ml plasma equivalents was given (two flasks of batch 226). Given a normal Factor IX turnover, a value approaching 50 per cent could have been expected 24 hours after starting substitution. The value found (<30 per cent) would seem to suggest an increased turnover at an increased body temperature (38°C) due to extensive contusions and grazing-wounds. It is not inconceivable, however, that the activity of batch 226 diminished during 14 month's storage at –25°C; the activity was not determined again after storage. In the further course of substitution with batch 260, Factor IX minimum values were found to be about 20 per cent at a daily single dose of 420 ml plasma equivalents (about one-third of the virtual distribution volume); these values are in accordance with expectation given a normal Factor IX turnover (half-life 20 hours); the patient was considerably better and afebrile during this period (fig. 5).

Side effects. Even in administration of PPSB in physiological saline, considerably diluted and

with small amounts of heparin added, the needle had to be inserted at different sites twice daily in the first three patients because an often very painful and sometimes rapidly ascending *thrombophlebitis* developed at the infusion site within 12 hours. Only in patient I 125/66 was it possible to leave the needle *in situ* up to 24 hours, once 10 mg non-esterified hydrocortisone was added daily; this medication greatly reduced the severity of phlebitic symptoms. All vessels returned to patency after cure of the thrombophlebitis. No phlebitic symptoms were observed in the boy, who received a single 30-minute infusion daily of PPSB diluted 1:5 with physiological saline.

In patient I 859/65, a causal relation between PPSB and *hepatitis* is possible but was not established with certainty, for this patient received fresh plasma transfusions also. In patient I 125/66 the relation is very doubtful because the interval between completion of substitution and the onset of hepatitis was over 6 months, during which period this patient received several transfusions.

The small amounts of heparin given (up to 20 mg daily) do not disturb haemostasis because they are too small to affect the mechanism of coagulation.

Discussion

Patients with a Factor IX deficiency with a blood concentration less than 20 per cent of normal, can develop vitally dangerous haemorrhages in surgical interventions and after severe injuries.

This indicates that the *haemostatically safe Factor IX concentration is higher than 20 per cent.*

Securing a safe blood concentration by administration of blood and plasma is possible only by exchange transfusion[8], certainly in the case of severe haemophilia B with a Factor IX concentration <1 per cent of normal. But in particular in patients who have received many transfusions, this entails appreciable risks. Plasmapheresis, like exchange transfusion, is anything but elegant and places considerably stress upon the patient as well as the personnel engaged in this treatment. It is therefore almost self-evident that administration of purified Factor IX is preferable by far to plasmapheresis and exchange transfusions. This is the more true because, in the event of complications of wound healing, sepsis, etc., the marked increase in Factor IX turnover increases the amount of Factor IX required to attain the haemostatically safe minimum concentration to proportions which exceed the scope of even intensive exchange transfusions[9].

Little is known with certainty about the amount of purified Factor IX required to ensure adequate haemostasis[1,8,16], not only because haemophilia B is uncommon and because a clinically valid Factor IX preparation has been available only a few years in the form of PPSB, but also because a *reliable Factor IX assay* has become available only recently. The one-stage method as we used it at our laboratory until 1962, yielded results with a coefficient of variation of at least 10 per cent[8]. The results obtained with the two-stage method so far used at many laboratories have a coefficient of variation as high as 25 per cent[1].

Only in 1963 did Veltkamp succeed – *by drastic improvement of the one-stage method*[3, 5,12,17] – in reducing *the coefficient of variation of the Factor IX activity found to 6-9 per cent.* This made the Factor IX assay sufficiently reliable for more exact metabolic studies and a safe control of substitution therapy. Factors of fundamental importance in improving the accuracy of Factor IX assay were: a) the use of the Schnitger coagulometer[14]; b) the use of a normal standard with constant Factor IX activity; c) the use of kaolin as activator of the contact phase of blood coagulation; d) adaptation of the dilutions of the test plasma, on the basis of the expected Factor IX activity, to dilutions of the standard, obviating the need for extrapolation in calculating the Factor IX activity to be assessed.

The newly developed reliable assay reinforced the foundation (laid in 1961) for calculating the amount of *purified Factor IX* required to ensure the haemostatically safe Factor IX concentration. The *biological half-life* of Factor IX was estimated as *20 hours* in a series of in part unpublished experiments[3]. This value closely approaches the rate of disappearance of purified Factor IX as established by other authors[11]. The *equilibration half-life* (measure of the rate at which the equilibrium between intravascular and extravascular Factor IX concentrations is attained) could not be determined with exactness. However, it seemed unusually short *(minutes)* and decidedly shorter than other investigators have reported[11]. Within 10 minutes of rapid administration of the starting dose of PPSB, only about half the dose administered was traceable intravascularly (see figs. 1

through 5). This agrees with previous personal observations[8] and with the findings obtained by the English group[1]; and it suggests that the *virtual distribution space* of Factor IX is *twice the intravascular plasma space*.

With all these data and hypotheses in mind, and on the basis of a previously presented equation[8], it is possible to calculate the *maintenance dose* (H) to be given daily by continuous drip as:

$$H = \frac{0.693 \times 2 \times p \times L \times 24 \times 1000}{t\frac{1}{2} \times 100} = 17 \times p \times L,$$

in which H = pure plasma equivalent in ml; 0.693 = nat.log. 2; 2 = multiplication factor for calculation of the virtual distribution space of Factor IX; p = plasma volume in litres; L = desired level of Factor IX in per cent; 24 = duration of infusion in hours; $t\frac{1}{2}$ = biological half-life of Factor IX.

The data compiled in Table III show that the Factor IX activities observed during substitution in actual practice correspond exceedingly well with the concentrations expected, i.e. calculated according to the above equation. These data lend strong support to the hypothesis of rapid distribution of the transfused Factor IX over a virtual space twice as large as the plasma space. Observations on the Factor IX concentrations during the final week of substitution in our patient with severe haemophilia B prove that this phenomenon is not a consequence of changes produced in Factor IX by the purification process. In this patient, we administered from the 6th through the 10th day an amount of PPSB equivalent to 720 ml pure fresh plasma (4 flasks of batch 254 at 180 ml equivalents per flask), and we continued the continuous drip for another $2\frac{1}{2}$ days with almost the same amount of unpurified Factor IX (800 ml pure fresh plasma). Throughout this period the Factor IX concentration remained virtually constant at 20 per cent (see fig. 4 and Table III: the value of the 6th day is still clearly influenced by the larger dose (1080 ml) of the preceding day).

The Factor IX concentration of a flask of PPSB in batch 254, therefore, corresponded with that of 180 ml pure fresh plasma, not only *in vitro* but likewise *in vivo*.

On the basis of the experience gained in our patients we venture to indicate the following general *dosage scheme* (Table IV).

TABLE IV: DOSAGE SCHEME FOR PPSB

	Factor IX dosage[1]
starting dose	800 x p[2]
1st through 4th day	17 x p x 40[3]
5th through 10th day	17 x p x 30
11th through 14th day	17 x p x 20

[1] The dosage is given in ml pure plasma equivalents per day

[2] p = plasma volume in litres

[3] 40, 30 and 20 indicate the Factor IX concentrations required during the respective periods of treatment, expressed as per cent of normal.

The dosage for patients with a mild type of haemophilia B is calculated by subtracting the basic concentration (pre-transfusion value) from the required concentration for each occasion.

Of course the treatment of haemophilia B is also subject to the rule that the PPSB dosage should be capable of being revised at any time in view of the results of Factor IX assays carried out twice daily. It must be borne in mind that the turnover of the clotting factor administered can increase[9], which calls for an increase of dosage.

Comparing the equations for calculation of the maintenance dose of Factor IX in haemophilia B with those concerning Factor VIII in haemophilia A[12], we find that they are identical, which demonstrates that the *turnover of Factor IX is the same as that of Factor VIII.*

The longer biological half-life of Factor IX (20 hours as against 14 hours for Factor VIII) compensates an unmistakably larger virtual distribution space (twice the intravascular plasma space for Factor IX, as against 1.4 times this space for Factor VIII). The *maintenance dosages* expressed in equivalents pure fresh plasma are accordingly the same for both types of haemophilia. The Factor IX dosage differs from the Factor VIII dosage only during the first 2-3 days: the period required for loading the distribution space in patients with haemophilia A (Factor VIII equilibrates considerably slower than Factor IX)[12].

For haemophilia A and haemophilia B alike, large amounts of concentrated clotting factor must be available for protection in the event of major surgical interventions; for example, for an adult with severe haemophilia B one must have the disposal of an amount of PPSB which in Fac-

tor IX activity is equivalent to 20 l plasma, and in the event of a complicated postoperative course the same amount again must be in readiness.

A few remarks may finally be made about the most economic and least inconvenient substitution procedure. In intermittent administration of PPSB the amount required to ensure a given minimum concentration is undoubtedly larger than in substitution by continuous drip, for in the former case the average quantity of PPSB degraded per unit of time is larger. For the patient intermittent administration is more convenient and less disagreeable, particularly because PPSB produces painful thrombophletibides at the site of infusion. We believe that, in intermittent administration, the injections must not be spaced less than 6 hours apart and the daily amount should be 25 per cent larger than the daily total given by continuous drip. In view of the possibility of serum hepatitis, the same precautions must be taken as in substitution therapy for haemophilia A[12].

Summary and conclusions

Since 1960 the Centre National de Transfusion Sanguine, Paris, has produced a plasma fraction called PPSB, which encompasses purified Factor IX suitable for the treatment of haemophilia B.

Eight patients with haemophilia B (3 with the severe, 1 with the moderate and 4 with the mild type) were treated by us with PPSB. Our experience in these eight cases would seem to warrant the following conclusions:

The biological activity of Factor IX in PPSB proves not to differ from that in fresh citrate plasma. The turnover rate of Factor IX is of the same order of magnitude as that of Factor VIII.

The amount of PPSB required to attain and maintain a haemostatically effective Factor IX concentration in the case of major operations or severe injuries is dependent on the plasma volume, the severity of the haemophilia and the degree of catabolism in the individual concerned. A loading dose of 80 per cent of the plasma volume raises the Factor IX activity by 40 per cent. The required daily maintenance dose in ml, provided it is given by continuous drip to a patient with normal catabolism, can be calculated by the formula:

17 x plasma volume (in litres) x required increase in Factor IX (in per cent).

During the first four days of substitution the Factor IX concentration must be maintained at 40 per cent; the goal during the 5th through 10th day should be 30 per cent; during the remaining four days a concentration of 20 per cent is probably sufficient. For an adult with severe haemophilia B this means a total amount of Factor IX equivalent to some 20 l pure plasma. The requirement may well be doubled in the case of a complicated postoperative course with increased catabolism. In mild and moderate types of haemophilia B the dosage is lower, dependent on the pretherapeutic Factor IX concentration. At intermittent injection of PPSB at 6-hour intervals the total dose should be about 25 per cent larger than the total required in continuous drip.

Side effects were not observed, apart from thrombophlebitis at the site of infusion of PPSB given by continuous drip. This phlebitogenic effect is unequivocally reduced by adding small quantities of non-esterified hydrocortisone to the infusion fluid.

Two instances of serum hepatitis of limited severity cannot be convincingly described as consequences of the PPSB substitution therapy.

Addendum

After submission of the paper, Dr. Soulier from the Paris "National Blood Transfusion Center" kindly informed us that none of the 13 other patients who have been treated with PPSB batch 226 have developed signs or symptoms of hepatitis.

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